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ABIOTIC AND BIOTIC EFFECTS ON SECONDARY SEXUAL TRAITS AT THE POPULATION AND INDIVIDUAL LEVELS IN A PASSERINE BIRD

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“We are surrounded by endless forms most beautiful and most wonderful, and it is no accident, but the direct cause of evolution by non-random natural selection – the only game in town, the greatest show on earth.”

-Richard Dawkins

ABSTRACT

Sexual selection is a major evolutionary force that may lead to changes in morphology, behaviour, mating systems, life history evolution and even speciation and extinction. In this thesis, I studied how abiotic and biotic factors affect fitness, and the expression and evolution of secondary sexual traits of pied flycatcher (*Ficedula hypoleuca*) individuals with variable dichromatic plumage colouration. I studied these questions using long-term data sets as well as manipulative and comparative approaches.

First, I studied whether the temperature-dependent breeding success of pied flycatcher males with different dorsal plumage colouration is due to the effects of the genotype of offspring or due to post-hatching paternal effects. Additionally, I investigated how increased or decreased parental effort affected future fitness of adult pied flycatchers, and the future expression of male sexual ornamentation. Furthermore, I examined the effects of environmental conditions experienced during the wintering season (in sub-Saharan Africa) on the expression of male secondary sexual traits, and the potential interactive effects between wintering conditions and male secondary sexual traits on future fitness of male and female pied flycatchers. Finally, I studied the possible effects of social environment on the development and signalling functions of male ornamental traits.

I found that offspring raised by black pied flycatcher males had lower body condition than offspring raised by brown males when it was cold, and that this temperature-dependent breeding success of males with different dorsal plumage colouration was due to post-hatching paternal effects. Moreover, I found a plastic response in melanin-based plumage colouration of young males experiencing brood enlargement so that males became darker in the following breeding season. On the other hand, females that experienced brood enlargement had a significantly lower local survival in the next breeding season than those females in the control or in the reduced treatment group.

I discovered that mean rainfall during the wintering season and winter NAO (North Atlantic Oscillation) index predicted both a plastic response and short-term microevolution on the wing patch size. The wing patch size of individuals decreased in winters of low precipitation and high NAO values, while in the same conditions individuals with a small wing patch seemed to suffer lower local survival than individuals with a large wing patch, and vice versa. I also found that males breeding in our study area became darker after dry and windy wintering conditions compared to moist and less windy wintering conditions. Moreover, after dry and windy wintering conditions females mated with large-patched males laid large clutches, whereas females mated with small-patched males laid small clutches after such wintering conditions. In addition, females paired with large-patched males after dry and windy wintering conditions had a higher probability of returning in the next breeding season than those that chose small-patched males after such conditions.

I reveal that the forehead patch size of pied flycatcher males affects the outcome of intraspecific male-male competition. Moreover, interspecific male-male competition between pied flycatcher males and collared flycatcher (*Ficedula albicollis*) males (a closely related sister species) seems to have led male pied flycatchers to resemble collared flycatcher females. This most likely leads to reduced aggression from more dominant collared flycatcher males.

The results of this thesis provide important novel information on how changing environment affects phenotype expression and evolution as well as phenotype-dependent selection in the pied flycatcher. It seems to be that to a great extent the individual, population and year variations of expressed male secondary sexual traits of pied flycatchers are maintained by fluctuating environmental conditions experienced during the breeding and wintering seasons. Thus, global climate change can affect the population dynamics of long-distance migratory species in many and various ways. Climate change can take very different directions on the two different continents where breeding and wintering take place. Such environmental changes may have strong influences on the direction of sexual selection, on the expression of secondary sexual traits and on geno-/phenotype-dependent fitness, and all these processes shape populations.

TIIVISTELMÄ

Sukupuolivalinta on voimakas evolutiivinen voima, joka voi vaikuttaa eliöiden rakenteisiin, käyttäytymiseen ja elinkiertoekologiaan sekä jopa lajiutumis- ja sukupuuttoon-kuolemisprosesseihin. Tässä väitöskirjassa tarkastelin, miten eloton sekä elollinen ympäristö vaikuttavat toissijaisten sukupuoliominaisuuksien ilmentämiseen ja evoluutioon kirjosiepolla (*Ficedula hypoleuca*). Tutkin näitä kysymyksiä käyttäen sekä pitkäaikaisaineistoja että manipulatiivisia ja vertailevia lähestymistapoja.

Ensiksi tutkin, johtuuko eriväristen kirjosieppokoiraiden lämpötilasidonnainen lisääntymismenestys eroista poikasten perimässä vai eroista eriväristen koiraiden jälkeläishoidossa. Lisäksi tutkin, miten kasvatettu tai pienennetty poikuekoko vaikutti aikuisten lintujen tulevaan kelpoisuuteen sekä koiraiden toissijaisten sukupuoliominaisuuksien ilmentämiseen seuraavana lisääntymiskautena. Seuraavaksi tarkastelin talvehtimisalueiden (Saharan eteläpuoleinen Afrikka) ympäristöolosuhteiden vaikutusta koiraiden toissijaisten sukupuoliominaisuuksien ilmentämiseen, sekä mahdollisia talvehtimisolosuhteiden ja koiraiden fenotyypin välisiä yhdysvaikutuksia koiras- ja naaraskirjosieppojen kelpoisuuteen. Lopuksi tutkin sosiaalisen ympäristön mahdollisia vaikutuksia koiraiden toissijaisten sukupuoliominaisuuksien kehittymiseen.

Kylmällä säällä mustien koiraiden kasvattamat poikaset olivat kevyempiä kuin ruskeiden koiraiden kasvattamat poikaset. Tämä eriväristen koiraiden lämpötilasidonnainen lisääntymismenestys johtui eriväristen koiraiden eroista hoitaa poikasia eri lämpötiloissa. Lisäksi löysin yksilönsisäisen vasteen melaniiniväri tyksessä niillä koirailla, joiden poikuekokoa oli kasvatettu niin, että koirasyksilöt tummenivat seuraavana lisääntymiskautena. Toisaalta, niillä naarailla, joiden poikuekokoa oli kasvatettu, oli pienempi todennäköisyys palata seuraavalle lisääntymiskaudelle kuin niillä naarailla, joiden poikuekokoa oli pienennetty tai ei ollut muutettu.

Talvehtimiskauden keskisademäärä sekä talvi-NAO (North Atlantic Oscillation) vaikuttivat koirasyksilöiden siipilaikun kokoon sekä siipilaikun koosta riippuvaan valintaan. Yksilöiden siipilaikun koko pieneni vähäsateisina korkean NAO:n talvina. Samojen olosuhteiden aikana pienilaikkuisilla koirailla oli alhaisempi elossasäilyvyys kuin suurilaikkuisilla koirailla, kun taas kosteiden, alhaisen NAO:n, talvina tilanne oli päinvastainen. Lisäksi koiraat sulkivat tummemman höyhenpuvun kuivien talvehtimisolosuhteiden aikana verrattuna kosteisiin talvehtimisolosuhteisiin. Havaitsin myös, että naaraat, jotka pariutuivat suurisiipilaikkuisen koiraan kanssa kuivan talven jälkeen munivat suurempia munalukuja kuin ne, jotka pariutuivat pienilaikkuisen koiraan kanssa. Lisäksi havaitsin, että kuivan talven jälkeen niillä naarailla, jotka pariutuivat suurisiipilaikkuisen koiraan kanssa, oli korkeampi elossäsäilyvyys kuin niillä, jotka pariutuivat pienilaikkuisen kanssa.

Tulosteni mukaan otsalaikun koko vaikuttaa lajinsisäisen koiras-koiraskilpailun lopputulokseen niin, että suurilaikkuiset koiraat olivat pienilaikkuisia aggressiivisempia. Lisäksi näyttäisi siltä, että lajienvälisessä koiras-koiraskilpailussa kirjosieppokoiraiden ja sepelsieppokoiraiden (*Ficedula albicollis*, sisarlaji) välillä ruskeat kirjosieppokoiraat mimikoivat sisarlajinsa naaraita. Tämä todennäköisesti vähentää sepelsieppokoiraiden aggressiivisuutta kirjosieppokoiraita kohtaan.

Väitöskirjani tulokset tarjoavat uutta tärkeää tietoa siitä, miten muuttuva ympäristö vaikuttaa fenotyypin ilmentämiseen ja evoluutioon sekä fenotyypistä riippuvaan valintaan kirjosiepolla. Tulosteni perusteella vaikuttaa siltä, että yksilön- ja populaationsisäistä sekä vuosien välistä vaihtelua toissijaisten sukupuoliominaisuuksien ilmentämisessä ylläpitävät suurelta osin vaihtelevat ympäristöolosuhteet sekä lisääntymis- että talvehtimisalueilla. Siten mailmanlaajuinen ilmastonmuutos voi vaikuttaa pitkänmatkan muuttolajin populaatiodynamiikkaan monella eri tavalla. Ilmasto voi muuttua hyvin eri tavalla kahdella eri mantereella, jolla lisääntyminen ja talvehtiminen tapahtuvat. Tällaiset ympäristönmuutokset voivat vaikuttaa hyvin voimakkaasti sukupuolivalinnan suuntaan, toissijaisten sukupuoliominaisuuksien ilmentämiseen sekä fenotyypiltään erilaisten yksilöiden kelpoisuuteen, ja kaikki nämä prosessit osaltaan muokkaavat populaatioita.

CONTENTS

LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts, referred to in the text by their Roman numerals:

I Järvistö PE, Calhim S, Schuett W, Velmala W, Laaksonen T. 2015. Foster, but not genetic, father plumage coloration has a temperature-dependent effect on offspring quality. *Behavioral Ecology and Sociobiology*, 69, 335–346.

II Järvistö PE, Calhim S, Schuett W, Velmala W, Laaksonen T. 2016. Sex-dependent responses to increased parental effort in the pied flycatcher. *Behavioral Ecology and Sociobiology*, 70, 157–169.

III Järvistö PE, Calhim S, Schuett W, Sirkiä PM, Velmala W, Laaksonen T. 2016 Carry-over effects of conditions at the wintering grounds on breeding plumage signals in a long-distance migratory bird: Phenotypic plasticity and selection. *Journal of Evolutionary Biology*, 29, 1569–1584.

IV Järvistö PE, Laaksonen T. The relationship between male sexual ornament and female fitness depends on environmental conditions experienced during the non-breeding season in the pied flycatcher. *Manuscript*.

V Calhim S, Adamík P, Järvistö PE, Leskinen P, Török J, Wakamatsu K, Laaksonen T. 2014. Heterospecific female mimicry in *Ficedula* flycatchers. *Journal of Evolutionary Biology*, 27, 660–666.

VI Järvistö PE, Laaksonen T, Calhim S. 2013. Forehead patch size predicts the outcome of male-male competition in the pied flycatcher. *Ethology*, 119, 662–670.

1. INTRODUCTION

Sexual selection is thought to be responsible for some of the most conspicuous ornamental traits found in animal taxa ranging from tiny invertebrates to large vertebrates (Andersson 1994). These traits, known as secondary sexual traits, are usually expressed only in one sex and are not directly a part of the reproductive system (Darwin 1871, Andersson 1994). Usually, it is the males that possess such conspicuous or colourful ornamentation (Darwin 1871, Andersson 1994). Sexual selection is a major evolutionary force that may lead to changes in morphology, behaviour, mating systems, life history evolution and even speciation and extinction (Andersson 1994). It has traditionally been assumed to operate in the opposite direction to natural selection (Darwin 1871, Fisher 1930), but nowadays it has been recognised to be a part of natural selection as it leads to a higher fitness in individuals (Andersson & Simmons 2006, van Doorn et al. 2009). Sexual selection can be divided into two major forms: intra- and intersexual selection. Intersexual selection operates through mate choice, while intrasexual selection functions through direct competition among individuals for access to mates, i.e. male-male competition (Darwin 1871). Even though the concept of sexual selection originates from Darwin's theory (1871), the intensive study of it did not begin until the 1970s (e.g. Williams 1975, Maynard Smith 1978). To date the topic of sexual selection has remained popular and countless numbers of fascinating studies have therefore accumulated, which allows raising even more complicated study questions.

1.1. Models of mate choice

According to sexual selection models, the choosy sex (usually females) prefers mates with conspicuous secondary sexual traits, such as plumage coloration and ornamentation (Darwin 1871, Fisher 1930, Andersson 1994), but the benefits of mate choice based on these conspicuous traits are still debated (e.g. Qvarnström 2001, Puurtinen et al. 2009). However, the general idea is that secondary sexual characters indicate the genetic (Zahavi 1975, Hamilton & Zuk 1982, Iwasa et al. 1991) or phenotypic quality of the bearer (Heywood 1989, Hoelzer 1989, Kirkpatrick 1996, Iwasa & Pomiankowski 1999). Models of indirect benefits predict that mating with attractive males will produce male offspring with conspicuous sexual ornamentation, and female offspring that have a preference for such male traits ('Fisherian runaway' process, Fisher 1930, Lande 1981, Kokko et al. 2003), or offspring of a high viability and fitness (the 'good genes' model, Zahavi 1975, Hamilton & Zuk 1982, Iwasa et al. 1991). Models of direct benefits predict that the conspicuous sexual characters of males signal their access to resources or their parental skills (which can be non-heritable). Consequently, females should gain direct benefits for their offspring by

selecting highly ornamented males, for instance, through the intensive feeding behaviour of such mates (Hoelzer 1989, Price et al. 1993, Linville et al. 1998, Iwasa & Pomiankowski 1999). Two other types of mate choice models are sensory exploitation models and sexually antagonistic coevolution models (Kokko et al. 2003). The first ones predict that female sensitivity e.g. to a certain colour in a non-mating context may explain their preference for males expressing such colouration in their bodies (Endler & Basolo 1998, Kokko et al. 2003). The latter ones are based on sexual conflict assuming that females do not evolve preference for males, but instead a resistance towards them in cases where females suffer fitness costs from too frequent matings ('chase-away sexual selection', Holland & Rice 1998, Kokko et al. 2003).

1.2. Male-male competition

Mate choice may often be directed at the resource that is defended by a male, typically the quality of the territory (e.g. Alatalo et al. 1986, Balmford et al. 1992, Buchanan & Catchpole 1997). It may thus seem that females base their choice on some certain male traits, for instance, considerable ornamentation, but the actual target might be the resource, and males that exhibit the trait may possess the highest quality of resources. In such a situation, success in male-male competition determines the success in mate choice (Alatalo et al. 1986, Griffith & Pryke 2006). The *fighting ability hypothesis* stresses that conspicuous colours make superior fighters recognizable and are favoured for this reason (Rohwer & Butcher 1988). Thus, sexual ornamentation may function as honest signals of fighting ability (Rohwer 1982, Senar 2006). In that case, signal honesty may be assured through repeated tests of fighting ability, where cheating subordinates are revealed. On the other hand, male-male competition may lead to the development of female-like dull colouration (i.e. female mimicry) in order to function as a concealment of the sex of the competing male (phenomenon called as alternative reproductive strategy, Jukema & Piersma 2006, Sternalski et al. 2012).

1.3. Phenotypic variation

The extensive variation of phenotypes occurring within populations is a result of both individual plasticity (e.g. an individual corresponding to environmental variation) and genetic differences (Hartl & Clark 1997, Pigliucci 2001). One major challenge thus far has been to explain how phenotypic variance is maintained in natural populations, since variance is assumed to be depleted by directional selection acting on fitness-related traits (Byers 2005, Walsh & Blows 2009). Sexually selected traits especially seem to be more phenotypically variable compared to non-sexual morphological traits (Pomiankowski & Møller 1995). Moreover, secondary sexual traits are excellent targets for studying phenotypic variation as they are usually both heritable and highly condition-dependent, and thus indicate genetic or phenotypic quality of an individual (Andersson 1994). Distinguishing between the plasticity of phenotypic traits and

between-individual selection acting on them is crucial for comprehensive understanding of how and why the traits change in time, and further to understand the potential consequences of the ever-changing environmental conditions on population dynamics (Visser 2008, Peñuelas et al. 2013).

1.3.1. Individual plasticity

While between individual-variation in the expression of secondary sexual traits has been studied extensively (i.e. why individuals differ from each other, Bortolotti 2006), we still have a relatively poor understanding of the alterations in phenotypes occurring over time within individuals (i.e. individual plasticity, Hayes & Jenkins 1997, Piersma & Drent 2003, Westneat et al. 2015). Individual plasticity may signal the condition-dependence of the trait of interest, whereas individual consistency may signal a genetic control over the expression of the trait or the ability to cope with stressful environmental conditions during early development (Gienapp et al. 2008, Peñuelas et al. 2013). As secondary sexual traits play an essential role in reproduction in many animal species, these traits should be considered a potentially important mediator of the costs of reproduction (Höglund & Sheldon 1998). Being often highly condition-dependent, the expression of a secondary sexual trait requires resource allocation, and hence is in a conflict with other life-history traits (e.g. self-maintenance, and immunocompetence; Folstad & Karter 1992, Höglund & Sheldon 1998). Albeit costly, the production and expression of a secondary sexual trait is advantageous for an individual when fitness benefits exceed fitness costs (i.e. leading to increased breeding success). However, investing in some other aspect of reproduction may lead to a poorer quality of the secondary sexual trait, and hence a lower breeding success in the future. Some evidence exists for such inter-seasonal carry-over effects where costs of reproduction are related to the future expression of secondary sexual traits and breeding performance in birds (e.g. Gustafsson et al. 1995, Norris et al. 2004, Siefferman & Hill 2005). For example, American redstart (*Setophaga ruticilla*) males who bred late in the season, molted tail feathers during migration (which led to dull colouration), and not at the breeding grounds before the migration, as the early-breeding males did. Moreover, events and processes occurring during the non-breeding season may carry-over to affect events taking place during the breeding season. While a large proportion of studies on carry-over effects from the non-breeding season to the breeding season have concentrated on migration phenology (e.g. Marra et al. 1998, Norris et al. 2004, Both & Marvelde 2007, McKellar et al. 2013), only a few studies have investigated how such effects act on the expression of secondary sexual traits. For instance, a within-individual analysis revealed that the lusher the vegetation (estimated from the normalized difference vegetation index, NDVI) at the wintering grounds, the longer the tail ornamentation (moulted during the wintering season) of the trans-Saharan migratory barn swallow (*Hirundo rustica*, Saino et al. 2004).

1.3.2. Geno-/phenotypes in a fluctuating environment

One hypothesis explaining the existence of phenotypic variance is that different geno-/phenotypes are adapted to different environmental conditions. Thus, alternative geno-/phenotypes either occupy different habitats or share the same habitat but perform differently over time if environmental conditions vary (Kassen 2002). Consequently, temporal or spatial environmental heterogeneity may to some degree be able to maintain existing variation in secondary sexual traits through genotype-by-environment interactions (Qvarnström 2001, Hedrick 2006, Cornwallis & Uller 2010, Rodríguez et al. 2013). Thus, in addition to plasticity, the phenotypic composition may vary over time and/or space if different geno-/phenotypes vary in their survival or fecundity across the environmental gradient. As a result, selection on traits, whether sexual or non-sexual, may not be constant, since genes that enhance individual fitness in one environment may decrease it in another environment (genotype-by-environment interaction, Falconer 1981, Qvarnström 2001, Bell 2010, Cornwallis & Uller 2010). Sexual ornaments may therefore have developed to signal the individual's abilities to cope with alternative environments, and individuals with different characters may perform best under different environmental conditions (Kawecki & Ebert 2004, Byers 2005).

Sexual selection models assume that the choosy sex (usually females) mates non-randomly in regard to one or more secondary sexual traits, such as colourful patches or long spurs, of the other sex (Darwin 1871, Fisher 1930, Andersson 1994). This has led to the idea that the choosy sex should always gain by selecting a mate with a conspicuous secondary sexual trait (despite of potential shifts in environmental conditions, Robinson et al. 2012). However, the idea of fluctuating environmental conditions affecting many aspects of population dynamics is widely accepted (e.g. Root et al. 2003, Bellard et al. 2012). Even so, the understanding of the effects of such fluctuating environmental conditions on sexual selection (e.g. on its direction) is still limited. Shifts in the environmental conditions might induce sudden reversals in the relative quality or sexual attractiveness of mates (phenomenon known as 'ecological cross-overs', Spottiswoode & Saino 2010, Botero & Rubenstein 2012). This might cause alteration in the signalling functions of secondary sexual traits, and thus, the honesty of secondary sexual trait may vary depending on the environmental context (Cornwallis & Uller 2009, Spottiswoode & Saino 2010). Therefore, the fitness consequences of a female choice might vary according to the environmental conditions, and female choice might be context-dependent.

1.4. Colourful sexual ornaments in birds

Striking colourations expressed by several avian species are breath-taking. The beauty of such colours has inspired researchers as well as nature enthusiasts for centuries to wonder of the potential functions and evolution of colourful ornaments.

It has now been shown that plumage colouration can function, for instance, as threat signals in intrasexual competition during the breeding season or in intraspecific competition during the non-breeding season, as signals of individual presence, as signals of individual quality in mate choice, or as signals in breeding or behavioural strategies (Hill & McGraw 2006). Alternatively, cryptic colouration may conceal and protect individuals against predators, parasites or conspecific and/or heterospecific competitors (Hill & McGraw 2006). Melanin pigmentation is the most frequently found colouration across animal taxa. However, due to its complicated endogenous forming mechanisms the processes and functions of melanins are less clear compared to carotenoids, which are the second most prevalent pigments in the avian species (McGraw 2006). While the formation of carotenoid-based pigmentation has been shown to relate to individuals' nutrition and health state, the link between melanin-based pigmentation to these factors is not so straightforward (McGraw 2006, Roulin 2015). Melanins can be classified into two general categories; eumelanins and pheomelanins (McGraw 2006). While eumelanins yield dark colorations, pheomelanins are responsible for reddish hues (McGraw 2006). In vertebrates the expression of melanin coloration is usually heritable and under genetic control (Roulin et al. 2004, McGraw 2006, Roulin & Ducrest 2013). However, there is also evidence that environmental conditions have an effect on the expression of melanin colouration (e.g. Griffith et al. 1999, McGraw 2006, Fargallo et al. 2007, Roulin et al. 2008a). In several taxa from birds to fishes, melanin coloration covaries with a number of fitness related traits, such as offspring quality (Roulin 2004a), parental effort (Siefferman & Hill 2003), stress-linked factors (Kittilsen et al. 2009, Almasi et al. 2010), mate choice (Møller 1988), social dominance (Senar & Camerino 1998, Mafli et al. 2011), and habitat choice (Roulin 2004b, Dreiss et al. 2012). In addition, temperature and food availability have especially been shown to cause fitness differences among individuals with different melanin colouration (see Roulin et al. 2008b, Piault et al. 2009, Sirkiä et al. 2010, Jacquin et al. 2012). The lack of pigmentation (unpigmented white colouration), on the other hand, may form colour patterns as well (e.g. Pärt & Qvarnström 1997, Griggio et al. 2011). Such unpigmented plumage signals are likely to be energetically cheap to produce. Thus, the signal honesty (signal of individual condition or fighting ability) of such traits must then arise from other costs such as maintenance costs or social costs (Bortolotti 2006). In addition, variation in the UV reflectance (structural colouration) of plumage has been discovered in several avian species (Derim-Oglu & Maximov 1994, Siitari et al 2002, Prum 2006). Structural colouration is formed when a light wave hits a surface and part of the light is scattered in other directions (Prum 2006). Such colouration is essential to take into account as many birds are able to detect light at UV wavelengths (300–400nm, Bennett et al. 1996, Eaton & Lanyon 2003). Moreover, there are indications of the UV component of plumage being heritable and condition-dependent in numerous avian species (Keyser & Hill 1999, Doucet 2002, Siefferman & Hill 2005, Py et al. 2006, Peters et al. 2007).

1.5. Pied flycatchers as a model species

The pied flycatcher (*Ficedula hypoleuca*) is an insectivorous sexually dimorphic hole-nesting passerine. It has been widely studied from many aspects of its biology and life-history (Lundberg & Alatalo 1992). This intensive study history has created a comprehensive and exciting basis for delving further into more complicated and intriguing study questions. The pied flycatcher has a number of characters that make it an ideal study species. It breeds willingly in human-made nest boxes, and is not disturbed by frequent visits to the nest, manipulations of many kinds or handling the adults and nestlings (Lundberg & Alatalo 1992). However, probably the most fascinating character in this charming species is the great scale of variation occurring in the plumage colouration of males (Laaksonen et al. 2015).

Pied flycatchers are long-distance migrants that breed in most of Europe and western Siberia, and winter in sub-Saharan Africa. The species undergoes a partial pre-nuptial moult during the wintering season, during which males change into their conspicuous breeding plumage (e.g. body feathers and tertials are replaced, but tail feathers are replaced during the post-breeding moult at the breeding grounds, Lundberg & Alatalo 1992). The plumage traits of males vary greatly among individuals (Lundberg & Alatalo 1992, Laaksonen et al. 2015). First, the dorsal plumage of males varies from almost completely black to almost completely brown (Drost 1936, Lundberg & Alatalo 1992). The plumage colouration of males is heritable (reported h^2 values vary from 0.6 to 0.88, Alatalo et al. 1994, Grinkov 2000, Lehtonen et al. 2009a) and partly age-dependent: males become slightly darker (ca. 15–20 %) between the ages of 1 and 2 years (Lundberg & Alatalo 1992). Recently, it has been shown that dorsal plumage colouration predicts the temperature-dependent breeding success of differently coloured male pied flycatchers, with the result that the mortality of nestlings of black males was higher compared to that of nestlings of brown males when the weather was cold during the nesting period (Sirkiä et al. 2010). However, it has remained unresolved whether this difference in breeding success is due to the effects of the genotype of the offspring or due to post-hatching paternal effects. Second, males exhibit three white ornamental patches, in the forehead, wing and tail, which vary greatly in size among individuals. While high heritability has been reported for the forehead patch (Potti & Canal 2011), heritability for the wing and tail patches has not yet been studied. Moreover, the possible production and maintenance costs of these ornamental traits are poorly known (but see Ruiz-de-Castañeda et al. 2012). In the Finnish population, large wing patch size has been shown to be preferred by females (Sirkiä & Laaksonen 2009). In the Spanish population, large forehead patch size has been found to be under intersexual selection (Potti and Montalvo 1991), while in other European populations it seems that the size of the forehead patch is not a mate choice cue (Dale et al. 1999, Galvan & Moreno 2009). The signalling function of the tail patch remains unknown. Third, variation in the UV reflectance of the plumage has been discovered in pied flycatchers as well as in many other avian species (Derim-Oglu &

Maximov 1994, Siitari et al. 2002, Prum 2006). For pied flycatchers, the heritability and condition-dependence of the UV component are still not well known. However, it has been shown that males that arrive early at the breeding grounds have higher UV reflectance, suggesting the possible condition-dependence of this trait (Siitari & Huhta 2002), as early arrival date has been shown to have direct fitness effects on pied flycatcher males (Velmala et al. 2015). Examples of different plumage traits are represented in Fig. 1.

Fig. 1. Examples of variation in plumage traits expressed by pied flycatcher males

Pied flycatchers share habitats with their sibling species the collared flycatchers (*Ficedula albicollis*), in central Europe. It has been suggested that the brown plumage in male pied flycatchers, a derived trait, forms the ancestral dark male phenotype of *Ficedula* flycatchers (Sætre et al. 1997), which originated due to secondary contact with collared flycatchers in central Europe after the last ice age (Qvarnström et al. 2010, Sætre & Saether 2010). The incidence of brown pied flycatcher males in a population is higher the closer it is to the central European sympatric area (Røskaft et al. 1986, Lehtonen et al. 2009b, Laaksonen et al. 2015). This is due to the advantage of having brown plumage in interspecific male-male competition with the more dominant collared flycatchers (Gustafsson & Pärt 1991, Sætre et al. 1993, Alatalo et al. 1994), and favoured by conspecific female mate choice to reduce costly hybridization (Sætre et al. 1997). Sætre and colleagues (Sætre et al. 1997) revealed that female pied flycatchers living in allopatric populations tend to prefer dark pied flycatcher males,

while those living in sympatric populations preferred brown males. In addition, these researchers have shown (Slagsvold & Sætre 1991, Sætre and Slagsvold 1992, Sætre et al. 1993) that both sexes of both species behave towards brown intruders as if they were females: males entice and females behave aggressively. Therefore, this is a potentially complex female mimicry system, with a single mimic (brown pied flycatcher males), two potential models (collared and pied flycatcher females), and four possible operators (both sexes of both species). It has remained to be resolved which of the two potential models (collared or pied flycatcher female) the mimic (brown pied flycatcher male) resembles most.

2. AIMS OF THE THESIS

In this thesis, I studied how abiotic (e.g. temperature) and biotic (e.g. heterospecific and conspecific individuals) factors affect fitness (i.e. breeding success and viability) and the expression and evolution of secondary sexual traits of pied flycatcher individuals with variable dichromatic plumage colouration. I studied these questions using long-term data sets as well as manipulative and comparative approaches. The purpose of study **I** was to discover whether the temperature-dependent breeding success of pied flycatcher males with different dorsal plumage colouration is due to the effects of the genotype of offspring or due to post-hatching paternal effects. In study **II** I investigated how increased or decreased parental effort affected future fitness of adult pied flycatchers, and the future expression of male sexual ornamentation. In studies **III** and **IV** I examined the effects of environmental conditions experienced during the wintering season (in sub-Saharan Africa) on the expression of male secondary sexual traits, and the potential interactive effects between wintering conditions and male secondary sexual traits on future fitness of male and female pied flycatchers. In studies **V** and **VI** I investigated the possible effects of social environment on the development and signalling functions of male ornamental traits. Study **V** examined whether the brown plumage colouration of pied flycatcher males has developed to mimic female plumage colouration of the closely related sister species, the collared flycatcher, and thus whether the brown plumage colouration of pied flycatcher males living in sympatry with collared flycatchers could be an advantage in heterospecific male-male competition. In study **VI** I was interested in resolving whether conspecific male-male competition might have affected the development of the signalling function of the male forehead patch.

3. MATERIALS AND METHODS

3.1. Study species

I use the pied flycatcher as a model species in this thesis. In Finland, the species arrives at the breeding grounds in May and breeds from late May to early July. On average, males arrive at the breeding grounds before the females to select and defend a nest hole, onto which they attempt to attract a female (Lundberg & Alatalo 1992). The median clutch size is seven, and the length of the incubation period is about 13–16 days (Lundberg & Alatalo 1992). Nestlings fledge at 16–17 days of age. The amount of available insect prey and the prevailing weather conditions affect the condition and survival of fledglings (Sanz 1995, Siikamäki et al. 1998, Eeva et al. 2002). In addition to the pied flycatcher, its closely related sister species, the collared flycatcher, is used in study **V**. These species live in sympatry in central Europe. Collared flycatchers winter in sub-Saharan Africa as well, but in more eastern areas compared to pied flycatchers (Adamík et al. 2016). In the pied flycatcher natal site fidelity in Fennoscandian populations is particularly low (0.1–1.8% of nestlings return to their native population, Lundberg & Alatalo 1992). However, adults show relatively high breeding site fidelity in Fennoscandian populations (return rates of males: 20–49%, return rates of females: 7–15%, Lundberg & Alatalo 1992).

3.2. Study areas

One of the main study sites used in this thesis is situated in Southwest Finland, on the island of Ruissalo in Turku (60º 26'N, 22º 10'E). In addition, long-term data collected from a study site in Siuntio, southern Finland (60 º15'N, 24º 18'E), were used in study **IV**. In the interspecific study **V,** the samples of collared flycatcher females were collected in Hungary (47°43'N, 19°01'E) and the Czech Republic (49°38'N, 17°18'E). The experiment conducted for studies **I** and **II** was carried out in Ruissalo during the breeding season of 2012, and data for carry-over effects of that experiment were collected during the next breeding season 2013 for study **II**. Long-term data used in study **III** were collected in Ruissalo between 2004 and 2014. These data were also used in study **IV**, but additionally long-term data collected in Siuntio between 1983 and 2007 were also used. Interspecific data for study **V** on pied flycatchers were collected in Ruissalo during the breeding seasons in 2008 and 2011, and on collared flycatchers in Hungary and the Czech Republic during the breeding seasons of 2000 and 2003. The experiment conducted for study **VI** was carried out in Ruissalo during the breeding season of 2011. There were 230 nest boxes available for passerines (inner bottom area: 144 cm^2, entrance hole: 32 mm) in Ruissalo between

2007 and 2010, while between 2011 and 2014 the number of nest boxes in Ruissalo was 436. The study site of Siuntio had 160 nest boxes between 1983 and 2007. In Ruissalo, the dominant tree species are Scots pine (*Pinus sylvestris*) and oak (*Quercus robur*), while most of the area in Siuntio consists of coniferous forest with some deciduous trees.

3.3. Experimental designs

3.3.1. Cross-fostering and brood size manipulation experiment (BSM)

In order to distinguish between the effects of genotype of offspring and post-hatching paternal effects in environment-dependent breeding success in the pied flycatcher (study **I**), and to study the carry-over effects of parental effort on future fitness of adult pied flycatchers and on the expression of male sexual ornamentation (study **II**), a brood size manipulation (BSM) was conducted using cross-fostering between pairs of nests matched for clutch size and hatching date. The brood size manipulation was done when the chicks were three days old. Based on the final clutch size (number of eggs) I either enlarged or reduced the brood size by one nestling or maintained the brood size. If some of the eggs in a clutch had not hatched after two days or if some of the chicks had died before the brood size manipulation was conducted, those unhatched eggs or dead chicks were replaced by other chicks of the same age from non-experimental extra nests. Chicks from the same nest as the origin were marked by gently removing downy feathers, either from the feather tufts on the back or the head, making sure the assigned paired nests differed from each other by this marking. This was done in order to identify the origin of the chicks at the age of five days when they were ringed with uniquely coded aluminium rings. At the age of 12 days, the chicks' body mass and wing length were measured, and blood samples were taken from a wing vein for paternity analysis and for sex determination of the chicks. The numbers of dead and live chicks were monitored throughout the study and after fledging.

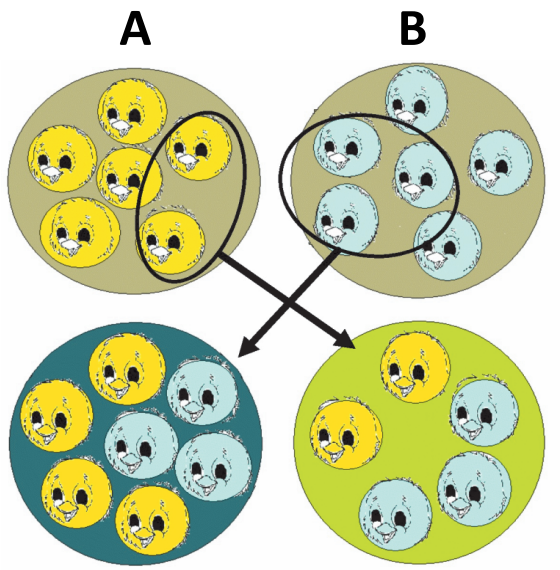

Fig. 2. An example of the cross-fostering / brood size manipulation procedure conducted for a pair of nests with an original brood size of six. As an outcome nest A ends up with seven chicks (three of which are originally from nest B), and nest B ends up with five chicks (two of which are originally from nest A).

3.3.2. Male-male competition experiment

To examine whether the size of the white forehead patch affects the outcome of male-male competition in the pied flycatcher (**VI**), I manipulated the forehead patch sizes of males using water-soluble colours and competed them against each other in a two-compartment aviary. The males were divided in two manipulation groups: small and large patches. Thus, each contesting pair consisted of a male with a large manipulated forehead patch and a male with a small manipulated patch. The two compartments were separated with a non-transparent removable wall. Each compartment was similar inside and had one nest box. Compartments were equipped with small trees and perches. A male with a manipulated large patch and a male with a manipulated small patch were introduced to their separate compartments immediately after the manipulation procedure and allowed to habituate for 60 minutes. Males with a small and a large forehead patch were placed in either the left or right compartments equally often, so as to avoid any possible position effects (Sætre & Slagsvold 1996). After an hour, the separation wall was removed and the two nest boxes were replaced with one nest box that was placed in the centre of the aviary. At the same time, a female was brought into the aviary in a small cage and placed on the floor below the new nest box. This was done to promote the competition between the males for the single nest box. Each contest lasted at least half an hour. Twelve contests lasted an hour, and six were interrupted before one hour had expired due to the intensive aggression of the

dominant male. Each contest was recorded with a video camera, positioned to face the nest box and the female's cage. All actions that occurred beyond the reach of the video camera were recorded by dictation. The number of attacks, chases, calls, songs, and perching on the female's cage, on the nest box or at the nest box hole were recorded.

3.4. Breeding data collection

Nest boxes were monitored weekly to record the laying date (pied flycatchers lay one egg per day), the clutch size, the hatching date, and the brood size at hatching. For studies **I** and **II** chicks were ringed at the age of five days (during the brood size manipulation year) and measured (body mass to an accuracy of 0.1g and wing length to an accuracy of 0.5mm) at the age of 12 days. For the other studies, chicks were ringed and measured between the ages of six and 13 days.

3.5. Phenotype data collection

Adult pied flycatchers were caught and ringed with aluminium rings during the breeding period. All individuals were aged as either young (1 year old) or old (≥ 2 years old) on the basis of their feather characteristics and the wear on their unmoulted wing coverts (Svensson 1992). Five different male ornamental traits were measured during 2004–2014 in the Ruissalo study site: the dorsal melanin colouration, UV reflectance and the sizes of the white forehead, wing, and tail patches. The proportion of black in the dorsal plumage of males was visually estimated either by Drost scores (Drost 1936) or by blackness percentages from 0 to 100%. Drost score I represents completely black males, while males belonging to score II have grey or brown rump. Intermediately coloured males belong to groups III-VI, while males displaying completely brown dorsal colouration belong to group VII (Drost 1936). The white patches of males were photographed with a millimetre scale in each picture in order to measure the sizes (in mm^2) of the patches using ImageJ software (http://rsb.info.nih.gov/ij/). To measure UV colouration, the partially white middle tertial feather was collected from each male. The UV reflectance was measured as the proportion of UV light, out of overall reflectance (R320-400nm/R320-700nm). A spectrophotometer (Avantes 2048, Avantes DH-S light source, Avantes, Eerbeek, The Netherlands) was used to measure the UV reflectance under standardised laboratory conditions. The feather was placed on top of a black non-reflecting cloth during the measurement. Three different points were measured from one feather of each male, and the average of these three values was used to represent the relative UV reflectance. In Siuntio, the measurement of the wing patch size was assessed as the proportion of white in the second tertial feather, which was estimated in 5% intervals. To be able to combine both these data sets (Ruissalo and Siuntio) in study **IV**, I standardised both data sets separately using the following formula (V – mean) / SD, where V is value, mean is mean of one data set and SD is the standard deviation of one data set. As a

result, the wing patch variable in the combined data set has a mean of 0 and a standard deviation of 1. In previous study (using the same data) Sirkä et al. (2010) assured that the proportion of white in the second tertial responded to the size of the wing patch by comparing the amount of white in the second tertial area to the whole white wing patch finding a strong positive correlation ($r_p = 0.54$, $P = 0.0002$, $N = 42$).

3.6. Environmental data

The prevailing temperature was used in study **I** to describe the local environmental conditions experienced during the breeding season. The temperature data were recorded 2 km from the study area, at the meteorological station in Artukainen in Turku (60°27'N, 22°10' E), and provided by the Finnish Meteorological Institute. Average temperatures for the nestling phases were determined individually for each nest. Three measurements of the environmental conditions at the wintering area were used in studies **III** and **IV**: the Normalized Differential Vegetation Index (NDVI, study **III**), the North Atlantic Oscillation index (NAO, study **III** and **IV**) and the amount of rainfall in the wintering area (study **III**). The wintering area of pied flycatchers breeding in Finland was identified using ring recoveries from sub-Saharan Africa (N = 3) and available geolocation data revealing locations of the wintering pied flycatcher males of our study population (N = 4, Ouwehand et al. 2015). Based on these data, pied flycatchers breeding in Finland winter in West Africa between 5.5° N and 11.5° N, and 6.5° W and 15.5° W (Fig. 3). The peak of the partial pre-nuptial moult of pied flycatchers takes place between mid-February and mid-March (Salewski et al. 2004). The Normalized Difference Vegetation Index (NDVI) describes the primary productivity of an area (Schmidt & Karnieli, 2002). The NDVI has been shown to correlate positively with biodiversity, especially with avian abundance (Osborne et al. 2001, Seto et al. 2004, Szép & Møller 2004). NDVI data recorded by NASA Earth Observations (NEO) were available at http://neo.sci.gsfc.nasa.gov. We calculated the mean February and March NDVI for the over-wintering region described above (Fig. 3). The winter (December–March) index of the North Atlantic Oscillation (NAO) is an indication of large-scale climatic variation (Hurrell 1995). Positive values are related to moist and relatively warm winters, while negative values are related to dry and cold winters in Europe (Hurrell 1995, Osborn 2006). The index has also been shown to describe environmental conditions in North and West Africa (e.g. Moulin et al. 1997, Oba et al. 2001, Evan et al. 2006). In contrast to Europe, positive NAO values correspond to drier winters and negative values to moister winters in Africa (Lamb & Peppler 1987, McHugh & Rogers 2001, Oba et al. 2001). In addition, the NAO index correlates positively with winds and dust activity in sub-Saharan Africa (e.g. Moulin et al. 1997, Evan et al. 2006). Winter NAO values were derived from http://www.cru.uea.ac.uk/~timo/datapages/naoi.htm. In addition, the amount of rainfall in the wintering area (Fig. 3) during the pre-breeding moult (from February to March) was used as an environmental predictor. Mean monthly rainfall values

were recorded by NASA Earth Observations (NEO) and obtained from http://neo.sci.gsfc.nasa.gov.

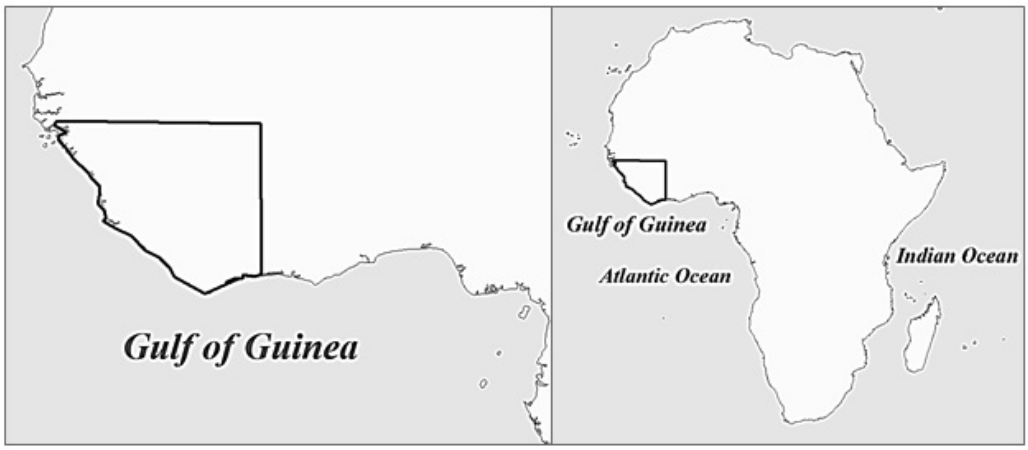

Fig. 3. The wintering area of pied flycatchers from a Finnish population on the basis of ring recoveries (N = 3) and geolocator data (N = 4) (Ouwehand et al., 2015). Plotted on ArcMap 10.2.2.

3.7. Statistical analyses

Linear mixed models were used to investigate the interactive effects of melanin-based phenotypes of the fathers, and the BSM and/or temperature on the body mass of the offspring (**I**). In these models, I used only foster-offspring in order to distinguish between genetic and post-hatching paternal effects. In addition, I used the rearing box as a random effect to control for pseudo-replication (because the chicks shared the same environment). When investigating the effects of the BSM on the direction of change in male ornamentation between two consecutive breeding years, mixed models for repeated data measurements were used (**II**). The probabilities of the experimental parental males and females returning in the following breeding season (local survival) were analysed using generalised linear models (**II**). To calculate repeatability estimates for male ornamental traits (**III**) and for female clutch size and preference for male wing patch size (**IV**), I used linear mixed models. Repeatability (V_I/V_P) captures the trait variance occurring among individuals (V_I) over the total phenotypic variance of the population (V_P). The remaining share ($1-[V_I/V_P]$) describes the amount of within-individual variation (Lessells & Boag 1987, Falconer & Mackay 1996). Following the recommendations of Nakagawa and Schielzeth (2010), individuals with only one or more measurements were included in the analyses. The variance components (estimated with restricted maximum likelihood) derived from the LMMs were used to calculate repeatability ($R = V_I/V_P$). Linear mixed models were used to test the effects of the environmental variables experienced during the wintering season on the expression of secondary sexual traits (**III**) or the interactive effects between such

environmental conditions and male ornamentation on the timing of breeding, the number of eggs laid, and the fledgling production (**IV**) at the population-level. To test for the individual-level changes, linear mixed models were run in a similar way to the population-level analysis, but only those individuals with more than one measurement (ornamentation: **III**, clutch size: **IV**) were included. Generalised linear mixed models with a binomial error structure were used to examine whether the probability of males surviving and returning to the breeding grounds the following breeding season was predicted by the measure of the secondary sexual trait, considering the environmental conditions experienced at the wintering grounds (**III**). Similarly was studied the probability of females surviving and returning in the following breeding season in relation to the size of the male wing patch and the environmental conditions experienced during the wintering season (**IV**). A Wilcoxon rank sum test was conducted on two independent samples for comparisons between plumage colourations of pied/collared flycatcher females and brown pied flycatcher males (**V**). This nonparametric version of the independent-sample t-test was chosen as the small sample size failed to meet its statistical assumptions. To examine the effect of the manipulated forehead patch on the outcome of male-male competition (**VI**) logistic regressions were used. Win-lose was held as a response variable and the manipulation treatment as an explanatory variable. In addition, pair identity was included into the model as a clustering factor to adjust d.f. for the paired design.

4. MAIN RESULTS AND DISCUSSION

4.1. Temperature-dependent effect on offspring quality (I)

The temperature during the nestling period influenced the body mass of foster-offspring of black and brown males differently. Foster-offspring raised by black males were lighter than offspring raised by brown males when it was cold. In contrast, when it was warmer foster-offspring raised by black males were heavier than offspring raised by brown males. On the other hand, colouration of the biological father did not interact with the temperature during the nestling period so as to affect offspring body mass. Moreover, the BSM did not have an interactive effect with the melanin colouration of the foster or biological father on foster-offspring body mass. The body mass of the foster-offspring of brown males varied less across the temperature gradient than for offspring of black males, suggesting that the breeding success of brown males is not as temperature-dependent as is that of black males. The melanin-based colouration of the biological father did not affect the condition of the foster-offspring in a temperature-dependent way. My results thus support the hypothesis that it is the behaviour of the male rather than the traits or genetic background of the chicks that might explain why there are temperature-related differences in the breeding success of black and brown males. The fact that differently coloured males did not differ in their breeding performance depending on the BSM treatment, further suggests that it might not be a question of brood demand or general stressfulness of parental duties that influences the offspring quality of differently coloured males, but specifically a response to temperature or a temperature-related factor. These findings are consistent with a long-term correlative study (where broods were not cross-fostered) conducted on pied flycatchers by Sirkiä et al. (2010). They found that the mortality of the nestlings of black males was higher than the mortality of the nestlings of brown males when the average temperature was below 14º C during the nestling period (Sirkiä et al. 2010). Together, theirs and my results suggest that when the average temperature during the nestling period varies from 14º C above 15º C, differences in breeding performance between brown and black males are expressed as variation in offspring condition. However, when it is colder (below 14º C), these differences are seen in offspring mortality.

4.2. Effects of increased brood size on male melanin colouration and female local survival (II)

I found an interactive effect between BSM and the breeding year (2012 and 2013) on melanin colouration, meaning that the annual change in dorsal blackness differed among individuals from the enlarged, reduced, and control groups. Unexpectedly, young males experiencing brood enlargement were clearly darker in 2013 than in 2012, while young males experiencing brood reduction or control procedure did not change

as strongly in their melanin colouration between years 2012 and 2013. Old males, on the contrary, did not change significantly in colour in any of the treatment groups. On the other hand, as old males are generally darker than young males (by ca. 15–20%) they might not become significantly darker than they already are. It has been shown that young pied flycatcher males are either not willing or able to take care of offspring as efficiently as older ones (Sætre et al. 1995, Laaksonen et al. 2011). The BSM-dependent change in the melanin colouration of young males indicates that an increase in the parental effort has an age-dependent effect on the complicated melanocortin system of pied flycatcher males. The pleiotropic effects of the melanocortin system cause melanin colouration to co-vary with several other physiological functions, for example with stress responses (Ducrest et al. 2008). Stress accelerates, for example, the secretion of the alpha-melanocyte-stimulating hormone and adrenocorticotropic hormone, which increases resistance to stressors (Khorram et al. 1985, Charmandari et al. 2005). My experiment revealed that males with enlarged broods increased their feeding frequency (see **I**). In addition, it has been shown that increased parental effort has elevated the heterophile:lymphocyte ratio of pied flycatcher males (Ilmonen et al. 2003). As elevated H:L ratio has been shown to have detrimental long-term effects on vertebrates (indicating a prolonged stress state), melanocortins might be targeting the immune cells even after the breeding event (Lobato et al. 2005, Kilgas et al. 2006, Davis et al. 2008). To reveal such a possible hormonal response, a thorough experiment would be required, where the stress levels of young and old male individuals would be manipulated prior to molting, after which the hormone levels and post-molt plumage colouration would be measured.

In addition, females experiencing a brood enlargement had a significantly lower local survival in the next breeding season than those females in the control or in the reduced treatment group. However, to the contrary, BSM did not affect the local survival of males in the next breeding season. My result indicates that females pay greater fitness costs than males when increasing their investment in the current reproduction. These fitness costs are paid through reduced residual reproductive value. In terms of breeding performance, male pied flycatchers would appear to benefit from having larger broods, whereas females would appear to suffer higher mortality. Thus, it seems that there is a sexual conflict concerning optimal brood size in this species. As the decision on clutch size is controlled by females (males having no direct influence on it), clutch size as a life-history trait has evolved to maximize the life-time fitness of females, reflecting the costs and benefits to them (Smith & Härdling 2000). As the egg formation process is energetically demanding for avian females (Monaghan & Nager 1997), females working over their optimal level might face energy constraints, which would lead, for example, to physiological or immunological stress (Alonso-Alvarez & Velando 2012). In addition, molt–breeding overlaps might have interacted with BSM in a complex way causing higher female than male mortality in enlarged broods. Moreover, males usually have a higher uncertainty of parentage, which may cause males to be more careful with parental care decisions (Sheldon & Ellegren 1998, Westneat & Stewart 2003).

Additionally, males may face a trade-off between caring for their current brood and searching for extra-pair copulation possibilities as well as new mates (Smith & Härdling 2000).

4.3. Effects of wintering conditions on the expression of the wing patch size and on the wing patch size-dependent survival of males (III)

I found that individuals were consistent in the expression of studied male traits (forehead and wing patches, UV reflectance and melanin colouration), but the strength of repeatability varied. The size of the *forehead patch* and *melanin colouration* had a strong repeatability (r = 0.70–0.72), while *UV reflectance* and the size of the *wing patch* had a moderate repeatability (r = 0.34–0.46). However, there was variation between years in all these traits at the individual- and population-level. The explanatory variables and processes behind this variation differed among the traits. Most intriguingly, mean rainfall and winter NAO index predicted both a plastic response and a potential short-term microevolution on the wing patch size. The wing patch size of individuals decreased in winters of low precipitation and high NAO values (i.e. drier and windier wintering conditions), while in the same conditions individuals with a small wing patch seemed to suffer lower local survival than individuals with a large wing patch, and vice versa. These effects of rainfall and NAO on plasticity and viability in relation to males' wing patch size revealed why within-individual changes along the rainfall and NAO gradient were masked at the population level (i.e. there was no relationship between winter NAO and male wing patch size at the population level). The survival-effect might be due to possible environment-dependent differences among individuals to adapt to prevailing conditions. On the other hand, the apparent survival-effect of winter NAO might as well originate from the conditions experienced during migration through Europe. Interestingly, a previous study has shown that during the breeding season, males with different sized wing patches differ in their nestling's survival rate in relation to precipitation; males with large patches suffer from higher nestling mortality than those with small patches during dry breeding conditions, and vice versa during moist breeding conditions (Sirkiä et al. 2010). Thus, it seems that different phenotypes are adapted to different environmental conditions prevailing during wintering and/or migration, as well as at the breeding grounds. Such a fluctuating selection at both ends of the flyway could potentially explain the maintenance of variation in this secondary sexual trait.

While the melanin-based dorsal colouration of pied flycatcher males was highly repeatable, I also found variation among the years in the expression of male melanin colouration at the population-level. A share of the annual variation in phenotypic composition of northern pied flycatcher populations has been explained by temperature-dependent breeding success of differently coloured males, and fluctuations in immigration from populations differing in melanin colouration (see Sirkiä et al.

2013). These new results show that another proportion of this variation could be explained by within-individual plastic changes induced by environmental conditions at the wintering grounds, as males breeding in our study area became darker after dry and windy wintering conditions compared to moist and less windy wintering conditions. Several studies attempting to explain variation in melanin colouration by e.g. variation in nutrition or parasite infections have failed to do so (e.g. McGraw & Hill 2000, McGraw et al. 2002, Senar et al. 2003, and also see e.g. Fargallo et al. 2007, Piault et al. 2012). Instead, the expression of the melanin colouration has been tightly linked to pleiotropy (Ducrest et al. 2008, Roulin 2016). Pleiotropic effects of the complex melanocortin system induce melanin colouration to covary with e.g. a response to stress (Charmandari et al. 2005, Ducrest et al. 2008). On the other hand, the darkening of male pied flycatchers might not only be due to pleiotropic side effects but also serve an adaptive purpose (see Dreiss & Roulin 2010). This could be the case, if in the years when there is a lower chance of survival males might invest more in breeding than in years of a higher chance of survival (i.e. individuals might invest differentially in breeding if colouration changes, see Dreiss & Roulin 2010).

4.4. Interaction between environmental conditions experienced during the wintering season and the size of male wing patch on fitness of a breeding pair, and female survival (IV)

I showed that while winter conditions did not interact with male wing patch size having an effect on laying date or fledgling production; it did have an effect on the clutch size. After dry and windy wintering conditions females mating with large-patched males laid large clutches, whereas females mating with small-patched males laid small clutches after such wintering conditions. After moist and less windy conditions, the situation was the opposite. This relationship is not influenced by the timing of breeding, and it is not due to individual females changing their clutch size in response to male wing patch size, as shown by within-female analyses; it is due to males pairing with females that lay different clutch sizes (that are highly repeatable). However, large-patched males after dry wintering conditions or small-patched males after moist conditions seem not to benefit from their success in attracting females that lay large clutches, as similar interaction between wintering conditions and male phenotype affecting fledgling production was not detected. Indeed, survival of fledglings is most likely to be strongly dependent on the amount of available insect prey and the prevailing weather conditions during the nesting period (e.g. Sanz 1995, Siikamäki et al. 1998, Eeva et al. 2002). Thus, the interactive effect between male phenotype and wintering conditions on the clutch size may disappear by the time of fledging. Moreover, I found a weak but significant negative correlation between winter NAO and the amount of rainfall at our study site during the following summer. Thus, winter NAO might have an effect on fledgling production of males with different sized wing patches as during a dry nesting period the mortality of offspring of large-patched males

is higher than that of small-patched males, and vice versa during a moist nestling period (Sirkiä et al. 2010). Alternatively, this might explain the disappearance of the interactive effect between male phenotype and winter NAO on the clutch size by the time of fledging.

According to the traditional view, choosing a mate with a large and conspicuous sexual ornamentation would lead to the highest possible fitness (Andersson 1994, Greenfield & Rodriguez 2004). However, to the contrary, I found that females paired with large-patched males after dry and windy wintering conditions had a higher probability of returning in the next breeding season than those that chose small-patched males after such conditions, and vice versa after moist and less windy wintering conditions. This relationship was independent of female clutch size and thus not a simple phenotypic correlation of clutch size and return rate. Therefore, a more likely explanation is that a large wing patch signals the male's condition after high NAO winters (i.e. dry and windy winters), while after low NAO winters (i.e. moist and less windy winters) the wing patch size does not signal the male's condition or the signal may even be reversed. Females experience fitness consequences specifically through the survival effect, as there was no male phenotype-dependent impact on fledgling production in relation to wintering conditions. Males in a good condition may carry out their parental duties more effectively than males in a poor condition, and thus females mated with such males do not need to compensate or work as hard as those mated with males in a poor condition. Indeed, I have shown that over-stressed pied flycatcher females (i.e. females experiencing brood enlargement) are less likely to survive into the next breeding season than females with decreased brood demand (i.e. females experiencing brood reduction (study **II**).

4.5. Heterospecific female mimicry (V)

There were differences between the melanin composition, and ultraviolet, and brown-red chroma in the plumage of pied flycatcher females and brown pied flycatcher males, while plumage traits of brown pied flycatcher males and collared flycatcher females were statistically indistinguishable. Thus, brown pied flycatcher males are more likely to be sexual mimics of heterospesific (collared flycatcher) rather than conspecific females. All previously reported cases of female mimicry in sexually dichromatic birds pertain to contexts where the (temporary or permanent) resemblance of the male mimic to the duller conspecific females functions as a defensive mechanism to minimise sexual recognition (Wiens 2001, Hawkings et al. 2012). Therefore, all members of these mimetic systems (mimic, model and operator) belong to the same species. These current results suggest that the model and the (main) operator are heterospecific to the mimic. Remarkably, none of the males of either species follow species assortative pairing patterns when given a choice (Sætre et al. 1997), despite the fact that perceptually the two females are (statistically) different. This suggests that at least in males, there is no ability or no selection for males that can distinguish between female

types or, by proxy, brown males from females. The high cost of erroneously displaying aggression towards a potential mate might explain the latter. In pied flycatcher females, this ability is also unclear, although one can assume that there is selection pressure to correctly indentify the sex of a brown individual, because nesting female pied flycatchers (but not female collared flycatchers) are rather aggressive towards brown 'intruders' (Slagsvold & Sætre 1991, Sætre et al. 1993). The abundance in sympatry of brown pied flycatcher males might represent an ongoing but spatially localised shift from dichromatism towards an apparent 'monochromatism' (or reduced phenotypic variation), which is favoured by a combination of strong agonistic and reproductive character displacement. Two general routes for the evolution of character displacement has been proposed (Rice & Pfenning 2007). According to one route, male brown colouration in pied flycatchers would be a trait that first appeared in sympatry (Sætre et al. 1997); according to the alternative route, brown plumage was already present as part of the phenotypic variation in pied flycatchers before secondary contact occurred. Because there is genetic variation in this trait, no genetic differentiation between sympatric and allopatric populations (Lehtonen et al. 2009b), and no evidence of a consistent selection against this trait in allopatry, the second route can better explain heterospecific female mimicry in *Ficedula* flycatchers.

4.6. Forehead patch size in male-male competitions (VI)

In my experimental study, males with a large forehead patch were significantly more aggressive than the males with a small patch in pair-wise competition. Thus, I show that the forehead patch size of pied flycatcher males affects the outcome of male-male competition. Being cheap to produce, the question of the honesty of white unpigmented signals is raised, as signals need to be honest in order to persist in a population (Zahavi & Zahavi 1997, Hanssen et al. 2006). Thus, to ensure reliability and evolutionary stability of the signal and to prevent dishonest information from cheaters, traits that signal fighting ability or the quality of the bearer have to be associated with costs (Maynard Smith et al. 1988). The costs of producing and maintaining conspicuous ornamentation may be related, for instance, to increased predation risk, direct energetic limitation, social punishments, increased vulnerability to feather-eating bacteria or the trade-off between immune competence and hormone activity (Møller 1987, Folstad & Karter 1992, Slagsvold et al. 1995, Nowicki et al. 1998, Hill 2011, Ruiz-de-Castañeda et al. 2012). However, as there is no evidence that a larger badge would increase the predation risk, at least, in the closely related sister species of the collared flycatcher (Pärt & Qvarnström 1997), and moreover, since the production costs of unpigmented badges are questionable (Rohwer 1982, Pärt & Qvarnström, Qvarnström 1997), I suggest that the high social costs may provide a mechanism which insures that the white forehead patch is an honest signal of fighting ability in the pied flycatcher. Males competing over a territory or a mate will benefit from signalling their true status, especially if there are frequent intrusions into territories by unknown males or by males

that do not remember the fighting ability of the resident (Rohwer 1982). However, as the cost of testing a large-badged individual is so high, it is possible that a small proportion of cheats that are never tested, may persist in a population (Evans & Hatchwell 1992).

5. CONCLUSIONS

The main conclusion of this thesis is that environment (abiotic and biotic) affects phenotype expression and evolution as well as phenotype-dependent selection in the pied flycatcher. Temperatures experienced during the nestling feeding period had different fitness (measured as offspring quality) effects on differently coloured male pied flycatchers (i.e. males with different proportions of melanin in their dorsal area, **I**). Moreover, the number of offspring taken care of during the breeding season (i.e. brood size) predicted age-dependent, within-individual change in the plumage darkness expressed during the subsequent season (**II**). Environmental conditions experienced during the wintering season, in turn, caused a within-individual plastic response in the expression of wing patch size and melanin colouration (**III**). These environmental conditions also predicted wing patch size dependent survival rate in male pied flycatchers (**III**) as well as interacted with the male wing patch size in predicting the clutch size of a breeding pair and the survival of females in the subsequent breeding season (**IV**). In addition, I have shown that it is most likely that the social environment during the breeding season influences the evolution of secondary sexual traits expressed in the pied flycatcher males (**V**, **VI**). As plumages of brown male pied flycatchers and female collared flycatchers were found to be indistinguishable (unlike plumages of female and brown male pied flycatchers), it is highly likely that the brown plumage of male pied flycatchers has evolved to mimic heterospecific females. By mimicking heterospecific females, brown pied flycatcher males can establish territories next to the more dominant collared flycatchers in sympatry. I also found that the white forehead patch of male pied flycatchers most likely signals the dominance in conspecific male-male contests, thus indicating that male-male competition has influenced the evolution of this conspicuous trait (**VI**).

It seems to be that to a great extent the individual, population and year variations of expressed male secondary sexual traits of pied flycatchers are maintained by fluctuating environmental conditions experienced during the breeding and wintering seasons. The abiotic environment (temperature) caused differences in the breeding success of males with different proportions of melanin in their dorsal area (**I**). Even though this difference was shown to be caused by the temperature-dependent variation in the parental behaviour of differently coloured males, the proportion of differently coloured males in the next generation is partly dependent on this temperature-dependent breeding success (see Sirkiä et al. 2013) as melanin colouration is heritable in this species (Lundberg & Alatalo 1992, Lehtonen et al. 2009b). Fluctuating environmental conditions experienced during the wintering season, in turn, caused variation in the phenotypic composition of populations through plastic within-individual responses, as well as through phenotype-dependent viability selection (**III**). Moreover, as weather conditions have been shown to have an influence on the brood

size decisions of pied flycatchers (e.g. Sanz 1995, Eeva et al. 2002), fluctuating environmental conditions experienced during the breeding season may indirectly affect melanin composition expressed by populations in the subsequent season through an induced age-dependent plastic response (**II**). On the other hand, it is not yet known how an abiotic environment affects the social environment (conspecific or heterospecific) of pied flycatchers. However, it is known that collared flycatchers (the more dominant species in sympatric areas) are more prone to harsh environmental conditions than pied flycatchers (Qvarnström et al. 2010). This explains why the overall breeding distribution of the pied flycatcher is more northern compared to that of the collared flycatcher (Qvarnström et al. 2010). In the case of fluctuating conditions experienced during the breeding season having an effect on the (conspecific or heterospecific) social interactions of the pied flycatchers, such conditions may by proxy affect the evolution of secondary sexual signals in male pied flycatchers.

The findings of this thesis are most likely not restricted to this single species, and such environment-dependent effects on geno-/phenotype expressions and evolution are probably to be discovered in several other species coping with ongoing environmental changes. There is evidence, for example, that global climate change is rapidly affecting the dynamics of natural populations (Walther et al. 2002, Peñuelas et al. 2013). Thus, in order to persist populations need to evolve new genetic adaptations for traits that improve fitness in an ever-changing environment through natural selection, in case the scope of their phenotypic plasticity is not sufficient (Parmesan 2006). Sexual selection is a part of natural selection as it operates on traits that improve mating success and hence fitness (Andersson 1994). Being a very powerful evolutionary force sexual selection may lead, together with anthropogenic environmental changes, to dramatic changes within populations. Global climate change can affect the population dynamics of long-distance migratory species in many and various ways (Walther et al. 2002). Climate change can take very different directions on the two different continents where breeding and wintering take place (e.g. Hulme et al. 2001, Christensen & Christensen 2003). Environmental changes may have strong influences on the direction of sexual selection, on the expression of secondary sexual traits and on geno-/phenotype-dependent fitness, and all these processes shape populations (Spottiswoode & Saino 2010, Roulin 2014). Whether genetic/phenotypic diversity decreases or increases, depends on which direction climate change takes and how populations are able to respond to the change (Spottiswoode & Saino 2010). In the case where the genetic/phenotypic diversity within populations decreases (e.g. due to strongly operating genotype/phenotype-dependent viability selection), the signalling functions of secondary sexual traits may weaken in the long term (Spottiswoode & Saino 2010). Moreover, current anthropogenic climate change can radically influence the honesty and information content of secondary sexual traits leading to ecological cross-overs (Botero & Rubenstein 2012, Greenfield & Rodriguez 2004, Spottiswoode & Saino 2010). In addition, phenotypic plasticity might sever the link between sexual ornamentation and male condition in the case where genotypes are expressed

differently in different environmental conditions (Spottiswoode & Saino 2010). Whether sexual selection is accelerated or hindered depends on which direction climate change takes. The next interesting step would be to investigate how current environmental changes affect biotic factors (e.g. brood size decisions and conspecific and heterospecific social environments), and whether these possible biotic changes influence the direction of sexual selection, evolution, and the expression of secondary sexual traits leading to shifts in population dynamics.

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